Nitrogen substrate–dependent nitrous oxide cycling in salt marsh sediments

by Qixing Ji1, Andrew R. Babbin2, Xuefeng Peng3, Jennifer L. Bowen4, and Bess B. Ward5

ABSTRACT

Nitrous oxide (N2O) is important to Earth’s climate because it is a strong absorber of radiation and an important ozone depletion agent. Increasing anthropogenic nitrogen input into the marine environment, especially to coastal waters, has led to increasing N2O emissions. Identifying the nitrogen compounds that serve as substrates for N2O production in coastal waters reveals important pathways and helps us understand their control by environmental factors. In this study, sediments were collected from a long-term fertilization site in Great Sippewissett Marsh, Falmouth, Massachusetts. The 15N tracer incubation time course experiments were conducted and analyzed for potential N2O production and consumption rates. The two nitrogen substrates of N2O production, ammonium and nitrate, correspond to the two production pathways, nitrification and denitrification, respectively. When measurable nitrate was present, despite ambient high ammonium concentrations, denitrification was the major N2O production pathway. When nitrate was absent, ammonium became the dominant substrate for N2O production, via nitrification and coupled nitrification-denitrification. Net N2O consumption was enhanced under low oxygen and nitrate conditions. N2O production and consumption rates increased with increasing levels of nitrogen fertilization in long-term experimental plots. These results indicate that increasing anthropogenic nitrogen input to salt marshes can stimulate sedimentary N2O production via both nitrification and denitrification, whereas episodic oxygen depletion results in net N2O consumption.

Keywords. Nitrous oxide, nitrogen substrate, nitrification, denitrification, salt marsh, sediment, long-term fertilization, 15N tracer

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1. Introduction

Nitrous oxide (N$_2$O) is a trace gas that has a strong greenhouse effect and is a powerful ozone depletion agent, with increasing emissions since the Industrial Revolution (Crutzen 1970; Cicerone 1987). Present-day N$_2$O concentration in the atmosphere is the highest it has been in the past 800,000 years (Schilt et al. 2010; Intergovernmental Panel on Climate Change [IPCC] 2013). With control of CFCs accomplished by the Montreal Protocol, N$_2$O is likely to be the single most important anthropogenic ozone-depleting agent emitted in the 21st century (Ravishankara, Daniel, and Portmann 2009).

Globally, more than 80% of total N$_2$O emissions can be attributed to microbial activities occurring in soil, open ocean, and coastal waters (IPCC 2013). Two microbial processes are the known major pathways for N$_2$O production. N$_2$O can be produced as a by-product during aerobic ammonium (NH$_4^+$) oxidation to nitrate (NO$_3^-$) by bacteria (Arp and Stein 2003) and archaea (Santoro et al. 2011). The other is denitrification, a stepwise reduction from NO$_3^-$ that emits N$_2$O as a free intermediate in the absence of oxygen. Thus, NH$_4^+$ and NO$_3^-$ are two nitrogen substrates for N$_2$O production. Generally, NH$_4^+$ is derived from organic nitrogen mineralization, whereas NO$_3^-$ is the product of nitrification, and the substrate for subsequent denitrification. Increasing anthropogenic nitrogen supply into coastal waters has led to excess NH$_4^+$ and NO$_3^-$, which are intercepted and removed by coastal wetlands and sediments at the interface between land and sea. The biological removal of excess NH$_4^+$ and NO$_3^-$ produces N$_2$O. Bange, Rapsomanikis, and Andreae (1996) estimated that coastal waters contribute up to 60% of global oceanic N$_2$O emissions. Salt marshes, situated in coastal areas, are “hot spots” of N$_2$O emission (Blackwell, Yamulki, and Bol 2010; Moseman-Valtierra et al. 2011).

Salt marshes are characterized by temporal and spatial variation of inorganic nitrogen (Brin et al. 2010) and oxygen availabilities (Howes et al. 1981). These two factors, combined with increasing nitrogen loading, regulate the magnitude and pathways of N$_2$O production. Determining the relative contribution of NH$_4^+$ and NO$_3^-$ to N$_2$O production can help us evaluate the relative importance of nitrification and denitrification to N$_2$O emissions in the environment. Oxygen critically affects N$_2$O production and consumption because (1) N$_2$O production via NH$_4^+$ oxidation requires molecular oxygen; (2) N$_2$O production from NO$_3^-$ and N$_2$O consumption are able to proceed only under low and zero oxygen; and (3) the enzyme that mediates N$_2$O consumption, nitrous oxide reductase (N$_2$OR), is the most oxygen-sensitive in the canonical denitrification pathway (Bonin, Gilewicz, and Bertrand 1989; Körner and Zumft 1989). Finally, N$_2$O emissions from salt marshes are expected to increase as a result of increasing anthropogenic nitrogen loading to coastal waters, as demonstrated by modeling and by nutrient enrichment experiments (Seitzinger and Kroeze 1998; Moseman-Valtierra 2012 and reference therein). Because of their natural gradients of oxygen and nitrogen concentrations, salt marshes are important experimental sites for studying N$_2$O production pathways and the effects of changing environmental conditions.
To investigate the effects of nutrient enrichment on salt marsh ecosystems, a long-term fertilization project was initiated in the 1970s in the Great Sippewissett Marsh, Falmouth, Massachusetts, by Valiela, Teal, and Sass (1973) and has been maintained without interruption. The results of long-term fertilization have included increases in aboveground biomass (Valiela, Teal, and Sass 1975; Howes, Dacey, and Goehringer 1986), loss of Spartina alterniflora and an increase in Distichlis spicata (Fox, Valiela, and Kinney 2012), and the alteration of microbial communities in high-nutrient environments (Hamlett 1986; Bowen et al. 2013). In addition, elevated rates of denitrification (Koop-Jakobsen and Giblin 2010; Kinney and Valiela 2013) and coupled nitrification-denitrification (Hamersley and Howes 2005) were associated with increasing fertilization.

In this study, biological N\textsubscript{2}O production and consumption in the Great Sippewissett Marsh sediments were investigated using \textsuperscript{15}N tracer incubation methods. Sediment NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−} were enriched with \textsuperscript{15}N, and the rates of \textsuperscript{15}NH\textsubscript{4}\textsuperscript{+} and \textsuperscript{15}NO\textsubscript{3}\textsuperscript{−} transformation to N\textsubscript{2}O were monitored over 8-hour incubations. The time courses were analyzed to determine potential rates of N\textsubscript{2}O production and to determine relative contributions of NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−} as substrates for N\textsubscript{2}O production. Furthermore, the effects of environmental factors, such as dissolved inorganic nitrogen availability, oxygen level, and fertilization level, on N\textsubscript{2}O production rates and the relative contribution of NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−} were investigated.

2. Methods
a. Site description and fieldwork

Sediment samples were collected from the Great Sippewissett Marsh located in Falmouth, Massachusetts (41°35′3.1″ N, 70°38′17.0″ W). Circular plots (10 m radius) of the marsh have been fertilized biweekly during the growing season (late April to early November, ∼20 weeks) without interruption since the early 1970s, using commercially available pelletized sewage sludge fertilizer (6% by weight total nitrogen, 0.9% NO\textsubscript{3}\textsuperscript{−}-N, 0.2% NH\textsubscript{4}\textsuperscript{+}-N; Milorganite, Milwaukee, WI). The fertilizer is applied at three levels (Table 1) to each set of replicate plots: low fertilization (LF), high fertilization (HF), and extrahigh fertilization (XF). Two additional plots are not directly fertilized above background and serve as controls (C). The plots are located within an area of 0.48 km\textsuperscript{2}, where averaged weekly background nitrogen loading from precipitation is estimated to be 0.023 g N m\textsuperscript{−2} week\textsuperscript{−1} (1.6 mmol N m\textsuperscript{−2} week\textsuperscript{−1}) (Bowen and Valiela 2001). Nitrogen loading from nitrogen fixation plus groundwater flow was 0.16 g N m\textsuperscript{−2} week\textsuperscript{−1} (11 mmol N m\textsuperscript{−2} week\textsuperscript{−1}) (Valiela and Teal 1979). At the time of sampling, the dominant vegetation cover in C, LF, and HF plots was short-form S. alterniflora in the high marsh and tall-form S. alterniflora in the low marsh. Low marsh in the XF plots was also dominated by tall-form S. alterniflora (XF-t). High marsh in the XF plots was dominated by D. spicata (XF-d) mixed with small patches of short-form S. alterniflora (XF-m). See Fox, Valiela, and Kinney (2012) for detailed maps of vegetation cover.
Table 1. Physical and chemical properties of sediment samples, including initial $^{15}$N percentage label for $^{15}$N-NH$_4^+$ treatment and $^{15}$N-NO$_3^-$ treatments. The concentrations of NH$_4^+$, NO$_3^-$, and N$_2$O in sediment are normalized to one gram of wet sediment. Standard deviations of measurements ($n = 3$) are shown in parentheses. The “n.d.” represents below detection [NO$_3^-$] in samples; therefore, $^{15}$N content of NO$_3^-$ could not be determined (represented as “-”). *Equivalent fertilizer loading in the unit of mmol-N m$^{-2}$ wk$^{-1}$.

<table>
<thead>
<tr>
<th>Plot</th>
<th>C</th>
<th>XF</th>
</tr>
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<tbody>
<tr>
<td><strong>Collection time</strong></td>
<td>August 2012</td>
<td>November 2012</td>
</tr>
<tr>
<td><strong>Fertilizer dosage</strong> (g N m$^{-2}$ week$^{-1}$)</td>
<td>0</td>
<td>7.8</td>
</tr>
<tr>
<td><strong>Moisture content</strong> (% total weight)</td>
<td>(80.6)</td>
<td>76.9</td>
</tr>
<tr>
<td><strong>N$_2$O (nmol g$^{-1}$)</strong></td>
<td>(0.48)</td>
<td>4.30</td>
</tr>
<tr>
<td><strong>NH$_4^+$ (nmol g$^{-1}$)</strong></td>
<td>270</td>
<td>263</td>
</tr>
<tr>
<td><strong>NO$_3^-$ (nmol g$^{-1}$)</strong></td>
<td>4.2</td>
<td>7.5</td>
</tr>
<tr>
<td><strong>$^{15}$N-NH$_4^+$ (%)</strong></td>
<td>0.419</td>
<td>0.420</td>
</tr>
<tr>
<td><strong>$^{15}$N-NO$_3^-$ (%)</strong></td>
<td>0.367</td>
<td>0.367</td>
</tr>
</tbody>
</table>
Sediment cores were collected in August 2012, November 2012, and August 2013 so that N₂O dynamics could be examined in summer and late autumn. The plots where sediments were collected during each trip are listed in Table 1. Three to four sediment cores, representing both high and low marsh habitat and primary plant types, were collected from C, LF, HF, and XF plots. After the roots were carefully removed, sediments from the same plot were homogenized, and subsamples were used for incubation. Therefore, the sediments do not represent N₂O production for a particular habitat but, to a certain extent, the entire fertilized or control plot as a whole. Such an experimental design minimized the effects of small-scale heterogeneity in this complex environment. Additionally, in November 2012, sediments representing different vegetation cover were collected from one of the XF plots. Sampling was performed at daytime low tide when the marsh bed was above water. Approximately 15 cm deep sediment cores were collected using 30 cm long, 7 cm diameter acrylic tubes with a sharpened edge. Butyl stoppers and rubber caps were used to seal the top and bottom of the acrylic tube storing intact sediment. The intact cores were kept in coolers with frozen reusable ice gel packs (Techni Ice, Frankston, VIC, Australia) for no more than 72 hours before conducting incubations.

b. Sediment incubations

Replicate sediment cores taken from the upper 10 cm of the same fertilized plots were homogenized and aliquotted (15 ± 0.2 g) into preweighed 30 mL amber serum bottles (Wheaton, Millville, NJ). The bottles were sealed with butyl rubber stoppers and aluminum seals (National Scientific, Rockwood, TN). Two sets of tracer amendments (5 mL injection) were applied. The ¹⁵N-NH₄⁺ treatments received ¹⁵N-labeled NH₄Cl (99%; Cambridge Isotope Laboratories, Tewksbury, MA) and natural abundance KNO₃ (Fisher Scientific, Pittsburgh, PA). The ¹⁵N-NO₃⁻ treatments received ¹⁵N-labeled KNO₃ (99%; Cambridge Isotope Laboratories) and natural abundance NH₄Cl (Fisher Scientific). The initial ¹⁵N labeling for NH₄⁺ and NO₃⁻ was usually <1% (Table 1). For NH₄⁺, the fraction of substrate ¹⁵N labeling was calculated, assuming the ¹⁵N content of the ambient NH₄⁺ in sediment to be close to natural abundance (¹⁵N/¹⁴N = 0.37%). The ¹⁵N labeling for NO₃⁻ was measured using the denitrifier method (Sigman et al. 2001). After the tracer solution was added, the bottles were vortexed with glass beads to distribute the tracers in the sediment. Incubations were performed under atmospheric oxygen headspace in order to simulate the surface sediment conditions because oxygen is likely to penetrate along with root matrices down to 10 cm. Incubation experiments lasted 6 to 8 hours at room temperature (22°C), during which triplicate samples were sacrificed every 2 hours by adding 1 mL of 7 M ZnCl₂ to terminate biological activity.

A subset of XF sediments from November 2012 incubated under helium headspace was compared with incubations of the same sediment under an oxygenated headspace in order to investigate the effect of oxygen on N₂O production and consumption. The headspace of the incubation bottles was adjusted to a lower oxygen level by purging with ultrahigh-purity
helium at 4 psi for 20 minutes. After oxygen adjustment, the sediments were incubated, and inorganic nitrogen concentrations were measured in triplicate bottles sacrificed every 2 hours by adding 1 mL of 7 M ZnCl₂.

c. Analytical methods

Sediment moisture content was determined from the difference between wet and dry weight after drying sediment samples at 65°C to constant weight. NH₄⁺ and NO₃⁻ were extracted from sediments using 2 M KCl after N₂O had been measured in the headspace. Slurries were placed on a reciprocal shaker for 30 minutes at 300 rpm, followed by centrifugation at 4,000 g. The supernatant was filtered (pore size 0.2 μm) and then frozen at –20°C until analysis. NH₄⁺ concentration was measured colorimetrically in triplicate using the phenol-hypochlorite method (Strickland and Parsons 1968); for 1 mL sample size, the detection limit was 0.5 μM. Samples with NH₄⁺ concentrations exceeding 50 μM and 500 μM were diluted 10- and 20-fold with 2 M KCl, respectively. Absorbance was measured on a UV-1800 UV-Visible Spectrophotometer (Shimadzu, Kyoto, Japan). NO₃⁻ concentration was measured using a hot (90°C) acidified vanadium (III) reduction column coupled to a Teledyne Chemiluminescence NO/NOx Analyzer (Model 200E) (Garside 1982; Braman and Hendrix 1989). Samples (20–100 μL) were injected in triplicate, with a detection limit of 0.1 μM.

Prior to headspace N₂O analysis, the serum bottle was vortexed so that N₂O in the sediment was equilibrated with the headspace. Thus, when N₂O was extracted from the headspace, it was considered to represent the concentration and isotopic composition of N₂O in the sediment. N₂O was extracted from the headspace using a 1 mL (XF samples) or 3 mL (C, LF, and HF samples) plastic syringe (BD Biosciences, San Jose, CA). Concentrations of N₂O in August and November 2012 were measured by electron capture gas chromatography (GC-8A; Shimadzu). The detection limit was 2 pmol N (25°C, 1 atm). N₂O concentrations for samples from August 2013 were measured by mass spectrometry (Delta V Plus; Thermo Scientific, Waltham, MA), with a detection limit of 0.1 nmol N. N₂O ¹⁵N/¹⁴N isotopic ratio (denoted as ¹⁵N-N₂O hereafter) was measured using mass spectrometry. Calibration standards for N₂O isotopic ratio, ranging from 0.37 to 1.74 ¹⁵N atom %, were prepared according to the denitrifier method (Sigman et al. 2001), assuming complete conversion of KNO₃ with known ¹⁵N/¹⁴N isotopic ratio.

The total amount of each nitrogen species was normalized to unit wet weight of sediment. Results were reported as nanomoles per gram of wet homogenized sediment (nmol N g⁻¹). This unit is equivalent to micromoles per liter, assuming the density of the sediment slurry was close to that of water. In the same manner, the N₂O production and consumption rates are reported as nanomoles per gram of wet homogenized sediment per hour (nmol N g⁻¹ h⁻¹). Such normalization on a mass basis rather than volume facilitated the computation of nitrogen transformation rates for comparison across different sediments.
**d. Model estimation of N$_2$O production and consumption rates**

Based on mass balance, a box model (Fig. 1) representing the processes of N$_2$O production from NH$_4^+$ ($r_{nit}$) and NO$_3^-$ ($r_{denit}$) and N$_2$O consumption ($r_{sink}$) was developed. NH$_4^+$ and NO$_3^-$ were the two major dissolved inorganic nitrogen species because NO$_2^-$ was below detection. Both NH$_4^+$ and NO$_3^-$ were considered as possible nitrogen substrates for N$_2$O. Even in incubations in the presence of atmospheric oxygen, significant decreases in N$_2$O concentration were observed; therefore, consumption of N$_2$O was also considered. Defined as the ratio of $^{15}$N over $^{14}$N transformation rates for a specific process, isotope effects ($\alpha$) associated with N$_2$O production from NH$_4^+$ ($\alpha_{nit}$), production from NO$_3^-$ ($\alpha_{denit}$), and N$_2$O consumption ($\alpha_{sink}$) were taken into account because the amended isotope comprised a very small fraction of the overall nitrogen pool. According to the compilations by Pérez (2005) and Dawson and Siegwolf (2007), isotope effects associated with N$_2$O production from NH$_4^+$ ranged from 0.932 to 0.965, production from NO$_3^-$ ranged from 0.97 to 0.99, and N$_2$O consumption ranged from 0.996 to 0.987 in pure culture, soil, and aqueous samples. The values of $\alpha_{nit}$, $\alpha_{denit}$, and $\alpha_{sink}$ in the model simulations were fixed at 0.96, 0.98, and 0.99, respectively. In the case of XF sediment from November 2012, varying the values of $\alpha_{nit}$, $\alpha_{denit}$, and $\alpha_{sink}$ by 0.03, 0.01, and 0.01, respectively, the percent changes in rates relative to the base case were <43%, and generally <30%, which fell within the standard...
Table 2. Descriptions of parameters used in numerical simulations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Unit</th>
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</thead>
<tbody>
<tr>
<td>$r_{nit}$</td>
<td>Rate of N$_2$O production from NH$_4^+$</td>
<td>nmol N g$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$r_{denit}$</td>
<td>Rate of N$_2$O production from NO$_3^-$</td>
<td>nmol N g$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$r_{sink}$</td>
<td>Rate of N$_2$O consumption</td>
<td>nmol N g$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$\alpha_{nit}$</td>
<td>Nitrogen fractionation factor for $r_{nit}$</td>
<td>Unitless, 0.96</td>
</tr>
<tr>
<td>$\alpha_{denit}$</td>
<td>Nitrogen fractionation factor for $r_{denit}$</td>
<td>Unitless, 0.98</td>
</tr>
<tr>
<td>$\alpha_{sink}$</td>
<td>Nitrogen fractionation factor for $r_{sink}$</td>
<td>Unitless, 0.99</td>
</tr>
<tr>
<td>N$_2$O</td>
<td>Sediment N$_2$O concentration</td>
<td>nmol N g$^{-1}$</td>
</tr>
<tr>
<td>$^{15}$NH$_4^+$</td>
<td>NH$_4^+$ $^{15}$N content</td>
<td>%</td>
</tr>
<tr>
<td>$^{14}$NH$_4^+$</td>
<td>NH$_4^+$ $^{14}$N content</td>
<td>%</td>
</tr>
<tr>
<td>$^{15}$NO$_3^-$</td>
<td>NO$_3^-$ $^{15}$N content</td>
<td>%</td>
</tr>
<tr>
<td>$^{14}$NO$_3^-$</td>
<td>NO$_3^-$ $^{14}$N content</td>
<td>%</td>
</tr>
<tr>
<td>$\frac{dN_2O}{dt}$</td>
<td>Change of N$_2$O concentration with time</td>
<td>nmol g$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$\frac{d(^{15}N_2O/^{14}N_2O)}{dt}$</td>
<td>Change of N$_2$O $^{15}$N content with time</td>
<td>% h$^{-1}$</td>
</tr>
</tbody>
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Equations (1) and (2) describe the changes in N$_2$O concentrations controlled by rates of production and consumption. These approximations will serve as a starting point for future in-depth investigation for isotope effects associated with N$_2$O production and consumption in salt marshes.

The N$_2$O production and consumption rates were prognostically modeled in MATLAB using equations (1) and (2), assuming constant rates during the incubation (see Table 2 for descriptions of parameters).

\[
\frac{dN_2O}{dt} = 0.5 \cdot (r_{nit} + r_{denit} - r_{sink}) 
\]

\[
\frac{d(^{15}N_2O/^{14}N_2O)}{dt} \approx \frac{1}{[N_2O]} \cdot \frac{^{15}N_2O}{^{14}N_2O} \cdot 0.5 \cdot (-r_{sink} \cdot \alpha_{sink} - r_{nit} - r_{denit} + r_{sink}) 
\]

\[
+ \frac{1}{[N_2O]} \cdot 0.5 \cdot \left( r_{nit} \cdot \alpha_{nit} \cdot \frac{^{15}NH_4}{^{14}NH_4} + r_{denit} \cdot \alpha_{denit} \cdot \frac{^{15}NO_3}{^{14}NO_3} \right) 
\]

Equation (1) assumes a mass balance on N$_2$O concentrations controlled by rates of production and consumption. Equation (2) describes the control of $^{15}$N-N$_2$O by rates of production and consumption, the $^{15}$N atom % of NH$_4^+$ and NO$_3^-$, and the isotope effects involved in N$_2$O production and consumption.
Model inputs are $^{15}$N content of NH$_4^+$ and NO$_3^-$, and $^{15}$N-N$_2$O measured at each time point. A grid search was performed for $r_{\text{nit}}$, $r_{\text{denit}}$, and $r_{\text{sink}}$ whereby the cost function was the mean-squared residual between the measured and the modeled values from N$_2$O concentration and isotope values from $^{15}$N-NO$_3^-$ and $^{15}$N-NH$_4^+$ treatments. The inverse of the standard deviation of the residual between modeled results and measurements was used as a weighting coefficient to normalize each cost component to similar magnitudes (see supplementary material available online). By assigning initial conditions listed in Table 2 and calculating the minimum cost function, the N$_2$O production rate from NH$_4^+$ ($r_{\text{nit}}$) and NO$_3^-$ ($r_{\text{denit}}$) and N$_2$O consumption ($r_{\text{sink}}$) were determined. To assess errors associated with modeled rates, a Monte Carlo simulation ($n=10,000$) was run for each incubation; the simulations were generated from Gaussian distributions with the same mean and variance as the measured data set.

3. Results

a. Sediment characteristics

Sediments collected from the top 10 cm of the marsh changed color with depth, from dark brown to black. Root matrices penetrated deeper than 10 cm, with C and XF sediment having the most (65% weight to weight ratio [w/w]) and least (26% w/w) vegetative materials. After the stems and roots of marsh vegetation were removed and the remaining sediment was homogenized, pore water content averaged $\sim$80% by weight (Table 1).

NH$_4^+$ concentration, in most cases, increased with fertilization level and in all plots exceeded NO$_3^-$ concentration. Within the same fertilized plot, NH$_4^+$ concentrations in August were generally lower than in November (Table 1). NO$_3^-$ concentrations were similar among C, LF, and HF plots, usually $<$8 nmol g$^{-1}$, but were two orders of magnitude higher ($>$500 nmol g$^{-1}$) in XF, XF-m, and XF-d sediment. In November 2012, XF-m and XF-d had $>$500 nmol N g$^{-1}$ NO$_3^-$, whereas XF-t had $<$5 nmol N g$^{-1}$ NO$_3^-$. The homogenized XF sediment (XF) consisted of 45% (w/w) of XF-m, 38% of XF-t, and 17% of XF-d and had NO$_3^-$ concentrations of $\sim$600 nmol N g$^{-1}$. Nitrite was not detected in any sediment samples. NH$_4^+$ plus NO$_3^-$ increased with increasing fertilization (Table 1). N$_2$O concentrations were similar ($<$1.2 nmol g$^{-1}$) in C, LF, and HF sediment, whereas XF sediment had elevated N$_2$O concentrations ranging from 4.3 nmol g$^{-1}$ in August 2012 to 177.3 nmol g$^{-1}$ in XF-d in November 2012.

b. Change of inorganic nitrogen with time during incubations of C and XF

The greatest contrast in the biogeochemistry in the salt marsh was observed between C and XF plots (Fig. 2); therefore, results from C and XF incubations are the focus of Sections 3b and c (for the complete data set, see Fig. S1 in the supplementary material). NH$_4^+$ concentrations in C incubations from November 2012 and August 2013 did not change significantly ($P = 0.18$ and 0.88, respectively, analysis of variance [ANOVA]) during the 8-hour incubation. XF sediments collected in August 2013 were the only incubation in which...
Figure 2. Time courses of $\text{NH}_4^+$, $\text{NO}_3^-$, and $\text{N}_2\text{O}$ concentrations during incubation experiments of C and XF sediments under atmospheric oxygen headspace. The concentrations of $\text{NH}_4^+$, $\text{NO}_3^-$, and $\text{N}_2\text{O}$ in sediment are normalized to one gram of wet sediment. Time zero indicates the moment of adding tracer solutions. (a) C sediment; (b) XF sediment. $\text{NH}_4^+$, $\text{NO}_3^-$, and $\text{N}_2\text{O}$ concentrations are shown in upper, middle, and lower panels, respectively.
NH$_4^+$ concentration showed a net increase after 8 hours. XF generally had higher initial NO$_3^-$ concentrations than C. Sediments from both C and XF plots that were incubated under atmospheric oxygen level had significant decreases in NO$_3^-$ concentrations, suggesting active denitrification. Net NO$_3^-$ consumption rates ranged from $\sim$0.1 nmol N g$^{-1}$ h$^{-1}$ in C from August 2013 to $\sim$80 nmol N g$^{-1}$ h$^{-1}$ in XF from November 2012 and even higher (>100 nmol N g$^{-1}$ h$^{-1}$) during the first 2 hours of incubation in XF from August 2013. Changes in N$_2$O concentration with time displayed different patterns with season. In November 2012, neither C nor XF incubations had significant change ($P > 0.1$, ANOVA) in N$_2$O concentrations, whereas in August 2013, N$_2$O concentrations decreased in both incubations. In the shorter incubation (3 hours) from August 2012, N$_2$O concentration decreased, but the trend was less pronounced.

c. Change of $^{15}$N – N$_2$O with time in $^{15}$N-NH$_4^+$ and $^{15}$N-NO$_3^-$ treatments during incubations of C and XF in November 2012

N$_2$O concentration did not change significantly in C and XF sediment; however, the increase in $^{15}$N$_2$O indicates N$_2$O production. In $^{15}$N-NH$_4^+$ treatments in November 2012, $^{15}$N-N$_2$O increased in both C and XF (middle panels of Fig. 3a and b). In $^{15}$N-NO$_3^-$ treatments, $^{15}$N-N$_2$O also increased in XF. In C $^{15}$N-NO$_3^-$ treatments, however, the $^{15}$N-N$_2$O pool was already enriched to 0.398 atom % (natural abundance = 0.367 atom %) at 30 minutes, but $^{15}$N-N$_2$O then decreased from 30 minutes to the end of the incubation (middle panels of Fig. 3a and b).

Both of these $^{15}$N-N$_2$O patterns in $^{15}$N-NO$_3^-$ treatments were also observed in other incubation experiments (Fig. S2b in the supplementary material), with low sediment NO$_3^-$ concentration correlating with decreasing $^{15}$N-N$_2$O and vice versa.

d. Modeled N$_2$O production rates

Model simulations of N$_2$O concentrations and N$_2$O isotope data were used to derive the N$_2$O production and consumption rates (Fig. 4). Even though there was no significant change in N$_2$O concentration in some cases (e.g., C and XF from November 2012 in Fig. 3), simultaneous N$_2$O production and consumption occurred in all experiments.

N$_2$O production rates from NH$_4^+$ were 0.05 $\pm$ 0.01 and 0.9 $\pm$ 0.1 nmol N g$^{-1}$ h$^{-1}$ in August 2012 C and XF sediment, respectively (Fig. 4a). N$_2$O production from NO$_3^-$ was greater than that from NH$_4^+$; rates were 0.2 $\pm$ 0.02 and 1.5 $\pm$ 0.2 nmol N g$^{-1}$ h$^{-1}$ in C and XF sediment, respectively. In November 2012, N$_2$O production rates from XF sediments ranged from 1.7 nmol N g$^{-1}$ h$^{-1}$ in XF-t to 55 nmol N g$^{-1}$ h$^{-1}$ in XF-d (Fig. 4b), one to two orders of magnitude higher than C, LF, and HF sediments, in which rates were generally <0.15 nmol N g$^{-1}$ h$^{-1}$ (Fig. 4c). When NO$_3^-$ was present at low to undetectable concentrations (C, LF, and HF), N$_2$O production was dominated by NH$_4^+$ oxidation; when NO$_3^-$ was abundant (XF, XF-d, and XF-m), N$_2$O production rates from NO$_3^-$ were 12–42 nmol N g$^{-1}$ h$^{-1}$, 3 to 15 times higher than N$_2$O production rates from NH$_4^+$, indicating
Figure 3. Time courses of N\(_2\)O concentration and \(^{15}\text{N}-\text{N}_2\text{O}\) during incubation experiment under oxygenated headspace. Time zero indicates the moment of adding tracer solutions. (a) C sediment from November 2012. (b) XF sediment from November 2012. Filled circles (from \(^{15}\text{N}-\text{NH}_4^+\) treatment) and triangles (from \(^{15}\text{N}-\text{NO}_3^-\) treatment) represent measurements; solid lines represent “best fit” of model simulation; shaded areas represent 95% confidence band for model simulations. Evolution of N\(_2\)O concentration in sediment is shown in the upper two panels; \(^{15}\text{N}-\text{NH}_4^+\) evolution in \(^{15}\text{N}-\text{NH}_4^+\) treatment and \(^{15}\text{N}-\text{NO}_3^-\) treatment is shown in the middle and lower panels, respectively. See Figure S3 (in the supplementary material) for the complete set of model simulations.

denitrification as the major production pathway. In XF-t where \(\text{NO}_3^-\) was low (~3 nmol g\(^{-1}\); Fig. S1 in the supplementary material) throughout the incubation period, unlike the other XF samples, N\(_2\)O production from \(\text{NO}_3^-\) was very low (<0.05 nmol N g\(^{-1}\) h\(^{-1}\)), and \(\text{NH}_4^+\) was the dominant source of N\(_2\)O, with a production rate of 1.7 ± 0.1 nmol N g\(^{-1}\) h\(^{-1}\). In August 2013 (Fig. 4d), XF sediment had much higher N\(_2\)O production rates than C and HF sediments. XF sediment had an initial \(\text{NO}_3^-\) concentration of 214 nmol g\(^{-1}\), but the
Figure 4. Modeled N$_2$O production rates from NH$_4^+$ (dotted bars), N$_2$O production rates from NO$_3^-$ (striped bars), N$_2$O consumption rates (gray bars), and contribution of denitrification to total N$_2$O production (black bars, scale on the right). Note that N$_2$O consumption rates are presented as negative values. (A) Sediment collected in August 2012. (B) Sediment collected in November 2012. (C) Rates of N$_2$O production and consumption and denitrification contribution in C, LF and HF incubations from November 2012. (D) Sediment collected in August 2013. (E) C, HF incubations from August 2013. Error bars represent Monte Carlo simulation ($n = 10,000$) derived standard deviations.
concentration dropped significantly to $<3 \text{ nmol g}^{-1}$ in the first 2 hours of incubation and maintained a low NO$_3^-$ concentration for the remainder of the incubation (Fig. 2b, middle panel). Thus, from 2 hours onward, nitrification became the major N$_2$O production pathway, at a rate of 11.7 nmol N g$^{-1}$ h$^{-1}$. N$_2$O production was mainly from NO$_3^-$ in C at a rate of $<0.03 \text{ nmol N g}^{-1}$ h$^{-1}$. Similar N$_2$O production rates occurred in HF, where both NH$_4^+$ and NO$_3^-$ served as nitrogen sources (Fig. 4e).

N$_2$O production rates from NH$_4^+$ and NO$_3^-$ were used to calculate relative contributions of nitrification and denitrification to N$_2$O production. The fraction of N$_2$O produced from denitrification in each experiment is shown in Figure 4. Except for C sediment collected in August 2013, all samples showed N$_2$O production from NH$_4^+$. In C, LF, HF, and XF-t sediment from November 2012, where NO$_3^-$ was low or undetectable, NH$_4^+$ was the major nitrogen source. When NO$_3^-$ was present ($>3 \text{ nmol g}^{-1}$), for example, in XF, XF-d, and XF-m from November 2012, the majority of N$_2$O was produced from NO$_3^-$.

e. Modeled N$_2$O consumption rates and the effect of oxygen on N$_2$O and NO$_3^-$ consumption

It is interesting to note that net N$_2$O consumption occurred in some incubations under atmospheric oxygen headspace, as shown by decreasing N$_2$O concentration (Fig. 2, lower panels; and Fig. S3 in the supplementary material). Sediments collected in August 2012 and 2013 showed greater N$_2$O consumption than production (Fig. 4a and d). N$_2$O consumption rates in November 2012 sediments were generally similar to or lower than production rates (Fig. 4b and c). N$_2$O consumption rates were higher in XF than in other plots; the lowest was 1 nmol N g$^{-1}$ h$^{-1}$ (XF-t from November 2012), and the peak was at $\sim40 \text{ nmol N g}^{-1}$ h$^{-1}$ in XF from August 2013. N$_2$O consumption rates for C, LF, and HF were generally $<0.2 \text{ nmol N g}^{-1}$ h$^{-1}$, one to two orders of magnitude lower than XF.

The subset of XF sediment from November 2012 incubated under helium headspace was compared with incubations under oxygenated headspace (Fig. 5). In helium headspace (−O$_2$) incubations, after NO$_3^-$ depletion, N$_2$O concentration decreased significantly to $<5 \text{ nmol g}^{-1}$ between 2.3 and 4.8 h, at a rate of $\sim25 \text{ nmol N g}^{-1}$ h$^{-1}$ (Fig. 5, upper panel). Lower N$_2$O consumption rate ($\sim15 \text{ nmol N g}^{-1}$ h$^{-1}$) was modeled when incubated with atmospheric oxygen (+O$_2$) in the presence of NO$_3^-$. No significant changes occurred in N$_2$O concentrations (Fig. 5, lower panel).

4. Discussion

Salt marsh sediment is potentially a net N$_2$O source to the atmosphere via nitrification and denitrification. These microbial processes depend on availabilities of NH$_4^+$ and NO$_3^-$ for nitrogen sources. Because NH$_4^+$ oxidation requires oxygen and NO$_3^-$ reduction does not, strong redox gradients in the sediment allow N$_2$O production via NH$_4^+$ oxidation, NO$_3^-$ reduction to N$_2$O, and N$_2$O consumption to co-occur. In Great Sippewissett Marsh,
sediment has been subjected to long-term fertilization, resulting in elevated $N_2O$ production and consumption rates.

a. Inorganic nitrogen availability controls nitrogen sources for $N_2O$ production

Results from incubation experiments showed that when $NO_3^-$ concentration was low or undetectable (e.g., C, LF, HF, and XF-t from November 2012), $NH_4^+$ was the dominant nitrogen substrate for $N_2O$ production, supported by the increase of $^{15}N$-$N_2O$ in $^{15}N$-$NH_4^+$ treatments (Fig. 3a). In $^{15}N$-$NO_3^-$ treatments, the decrease of $^{15}N$-$N_2O$ from initial $^{15}N$ enrichment indicated that $NH_4^+$, which was not labeled with $^{15}N$, contributed nitrogen to $N_2O$. Model simulations confirmed that $NH_4^+$ oxidation alone could explain the progressive enrichment of $N_2O$ with $^{15}N$ in $^{15}N$-$NH_4^+$ treatments, as well as dilution by $^{14}N$ from unlabeled $NH_4^+$ in $^{15}N$-$NO_3^-$ treatments (Fig. 3a). When $NO_3^-$ was present throughout the incubation experiment, for example, XF, XF-d, and XF-m sediment from November 2012
where $\text{NO}_3^{-} > 500 \text{ nmol g}^{-1}$ (Table 2), both $\text{NO}_3^{-}$ and $\text{NH}_4^{+}$ were nitrogen substrates for $\text{N}_2\text{O}$ production, as indicated by simultaneous increase in $^{15}\text{N}-\text{N}_2\text{O}$ in both $^{15}\text{N}-\text{NH}_4^{+}$ and $^{15}\text{N}-\text{NO}_3^{-}$ treatments (Fig. 3b). Model simulation showed that the major nitrogen source compound for $\text{N}_2\text{O}$ was $\text{NO}_3^{-}$, presumably as a consequence of active denitrification, consistent with the observed decrease in $\text{NO}_3^{-}$ concentration (Fig. 2b).

The nitrogen substrate determination provides insights into $\text{N}_2\text{O}$ production pathways. As a product of heterotrophic remineralization, $\text{NH}_4^{+}$ was always present at high concentrations ($> 70 \text{ nmol g}^{-1}$). In sediment with low $\text{NO}_3^{-}$ concentrations, such as C, LF, and HF sediments, $\text{NH}_4^{+}$ was the major substrate for $\text{N}_2\text{O}$ production. Thus, it appears that the $\text{N}_2\text{O}$ production pathways supported by $\text{NH}_4^{+}$ oxidation, such as nitrifier denitrification and coupled nitrification-denitrification (Wrage et al. 2001), are responsible. $\text{NO}_3^{-}$ could be supplied by fertilizer or potentially by nitrification (Kaplan, Valiela, and Teal 1979), or from groundwater discharge (Valiela and Teal 1979). Current incubation experiments showed denitrification was responsible for the majority of $\text{N}_2\text{O}$ production in sediments with high $\text{NO}_3^{-}$ concentration ($\sim 500 \text{ nmol g}^{-1}$), despite $\text{NH}_4^{+}$ being the major form of inorganic nitrogen. This suggests that $\text{NO}_3^{-}$ availability may be an indicator of $\text{N}_2\text{O}$ production pathways in these sediments, consistent with short-term $\text{NO}_3^{-}$ addition stimulating $\text{N}_2\text{O}$ emission as reported by Moseman-Valtierra et al. (2011). Further research could investigate the relative physiological advantage between nitrification and denitrification in these sediments under different $\text{NO}_3^{-}$ availabilities to test how $\text{N}_2\text{O}$ production pathways are regulated.

b. The control of $\text{N}_2\text{O}$ production and consumption by oxygen

Oxygen concentration, which is spatially and temporally variable in tidal marshes, regulates $\text{N}_2\text{O}$ production and consumption. When incubated under atmospheric oxygen headspace, $\text{NH}_4^{+}$ oxidation, $\text{NO}_3^{-}$ reduction, and $\text{N}_2\text{O}$ consumption were detected. In XF sediment from November 2012, $\text{N}_2\text{O}$ production was balanced by consumption, as indicated by no significant change in headspace $\text{N}_2\text{O}$ concentrations over the course of the incubation. The significant decrease in $\text{N}_2\text{O}$ concentration under low oxygen conditions suggested elevated $\text{N}_2\text{O}$ consumption and decreased $\text{N}_2\text{O}$ production, because once oxygen was removed from the headspace, $\text{NH}_4^{+}$ oxidation ceased so that $\text{NO}_3^{-}$ became the only possible nitrogen source for $\text{N}_2\text{O}$ production. As nitrification ceased and thus no more $\text{NO}_3^{-}$ was produced, denitrification resulted in a net loss of $\text{NO}_3^{-}$, and eventually $\text{NO}_3^{-}$ was depleted. Greater net consumption of $\text{N}_2\text{O}$ followed because (1) there was no $\text{NH}_4^{+}$ oxidation producing $\text{N}_2\text{O}$, (2) depletion of $\text{NO}_3^{-}$ prevented an additional $\text{N}_2\text{O}$ source, and (3) $\text{N}_2\text{OR}$ was relieved from oxygen inhibition; this was confirmed by the elevated $\text{N}_2\text{O}$ consumption rate discussed in Section 3e.

As the plants’ roots penetrate deeper than 10 cm, sediments collected for incubation experiments are likely to experience molecular oxygen supplied from tidal water as well as diffusion from air. In August, actively growing $\textit{S. alterniflora}$ oxidizes the sediment (Howes et al. 1981), potentially favoring the growth of aerobes and aerobic metabolism.
Because incubation experiments were performed in a closed system with limited oxygen, such active aerobic processes would lower the oxygen concentration in sediment, allowing N$_2$O consumption to occur. This could be the reason that N$_2$O consumption exceeded production during incubation in August (Fig. 4a, d, and e). In November, growth of *S. alterniflora* ceases and in situ remineralization supports the growth of denitrifiers. When incubation was performed in the absence of oxygen, NO$_3^-$ and N$_2$O were consumed in a few hours, indicating the presence of active denitrifiers. When oxygen was present in the headspace, oxygen inhibition of N$_2$OR decreased N$_2$O consumption rates. Further studies focused on the effects of oxygen availability on activities of nitrifiers and denitrifiers on a tidal cycle as well as seasonal cycle would provide more insight into N$_2$O fluxes in these time frames.

c. Nitrogen loading affects the N$_2$O production rates

As demonstrated in this study, increased N$_2$O production and consumption rates correlate with nitrogen loading from fertilizer. This is probably because fertilizer input enhances plant growth, and subsequent accumulation of above- and belowground biomass provides nutrients and electron donors to support nitrogen metabolisms, including denitrification (Hamersley and Howes 2005; Koop-Jakobsen and Giblin 2010). It should be noted that intermediate nitrogen loading (LF and HF plots), though already higher than the nitrogen loading of the vast majority of New England salt marshes, did not significantly increase sediment N$_2$O concentrations. Only XF plots, where the nitrogen loading is more than 40 times higher than background, showed significantly elevated N$_2$O concentrations. This indicates that elevated nitrogen loading at low to intermediate levels does not necessarily enhance N$_2$O production. Thus, denitrification is likely important in the consumption of N$_2$O. This is consistent with the finding of Lee et al. (1997) who showed that the emission ratio of N$_2$O:N$_2$ in marsh sediment is lower under higher nitrogen loading and called for future studies targeting the variability of N$_2$O:N$_2$ emissions in salt marsh sediment.

Increasing nitrogen loading from fertilization also resulted in changes in the relative contribution from NH$_4^+$ versus NO$_3^-$ to N$_2$O production. Fertilization results in higher NO$_3^-$ availability from fertilizer and possibly from NH$_4^+$ oxidation, thus increasing NO$_3^-$ availability to support NO$_3^-$ reduction to N$_2$O. As N$_2$O yield during denitrification is generally higher than that of nitrification (Bange 2008), it is likely that NO$_3^-$ reduction to N$_2$O becomes the major production pathway when denitrification occurs. This was observed in XF plots except XF-t from November 2012. In sediments with low fertilizer supply and in XF-t from November 2012, NH$_4^+$ was the dominant inorganic nitrogen form and NO$_3^-$ was present at low concentration because of rapid turnover by denitrification and tidal efflux (Brin et al. 2010). Therefore, the majority of N$_2$O was produced via NH$_4^+$ oxidation. Unlike all other XF samples, XF-t behaved more like the lower fertilized samples. NO$_3^-$ concentration was low in XF-t in November 2012 possibly because of its lower elevation, which is subjected to
more frequent tidal exchange. Thus, N$_2$O production was dominated by NH$_4^+$ oxidation; the production and consumption rate ($\sim$1 nmol N g$^{-1}$ h$^{-1}$) was an order of magnitude higher than rates in lower fertilization plots ($\sim$0.1 nmol N g$^{-1}$ h$^{-1}$).

Studies conducted in New England salt marshes (e.g., Great Sippewissett Marsh and Plum Island Estuary) have shown that decadal-scale elevated fertilizer input promotes the removal of excess nitrogen via denitrification (Hamersley and Howes 2005; Koop-Jakobsen and Giblin 2010). However, many adverse effects are reported, such as increasing NO$_3^-$ export to the adjacent estuary (Brin et al. 2010), subsidence of marsh surface because of reduction of organic matter accumulation (Turner et al. 2009), loss of marsh coverage because of reduced stability of sediment-root matrices and sea-level rise (Deegan et al. 2012), and greater N$_2$O production, as demonstrated in this study.

d. Factors in interpretation of experimental and model results in the actual environment

Homogenization of the sediment for these incubation experiments greatly altered the conditions to which organisms were exposed. Because this disruption occurred in all experiments, it should not obscure the treatment effect of different fertilizer levels. During the handling of sediment, availabilities of inorganic nitrogen might have changed because of active nitrification and denitrification. Exposing the sediment under atmospheric oxygen headspace may have partially inhibited denitrifiers, leading to underestimation of the reduction of NO$_3^-$ or N$_2$O, as well as overestimation of nitrification. Despite homogenization in atmospheric oxygen, however, both nitrifiers and denitrifiers were apparently still active in the incubations, as shown by the simultaneous increase of $^{15}$N-N$_2$O in both $^{15}$N-NH$_4^+$ and $^{15}$N-NO$_3^-$ treatments (Fig. 3b). Thus, $^{15}$N tracer incubation experiments identified potential pathways and rates for N$_2$O production and consumption. Also, incubation with homogenized sediment allowed the isolation of the intercorrelated variables, such as fertilization, plant biomass, and sediment inorganic nitrogen levels, which was necessary to discern the dependence of N$_2$O production on environmental factors.

The model simulation in this study is capable of distinguishing nitrogen source compounds for N$_2$O production but cannot determine the exact biochemical pathways involved. Even though there was coupling between nitrification and denitrification, during which nitrogen was transferred from NH$_4^+$ to NO$_3^-$ and to N$_2$O, the data did not specify the pathway by which the transfer occurred. It may be possible in the future to use the dual isotope approach, combining $^{18}$O as another tracer to identify the pathways (Kool, Van Groenigen, and Wrage 2011). The model parameterization in this study, using constant N$_2$O production and consumption rates, adequately described the majority of the experimental data. However, NH$_4^+$ oxidation, NO$_3^-$ reduction, and N$_2$O reduction are enzymatic processes, often assumed to follow Michaelis-Menten kinetics. The use of concentration-dependent nitrogen transformation rates may simulate the observations more accurately in the few incubations in which concentrations changed appreciably, notably the XF experiment in August 2013. In this experiment, the N$_2$O concentration decreased, which probably caused the rate of N$_2$O
consumption to decrease, and this was poorly represented by concentration-independent modeled rates (Fig. S3 in the supplementary material).

In C sediment from November 2012, N$_2$O was rapidly (i.e., by the first time point) enriched with $^{15}$N in the $^{15}$NO$_3$ treatment, whereas there was no initial $^{15}$N enrichment of N$_2$O observed in the $^{15}$N-NH$_4^+$ treatment. Similar observations have been reported by Stevens et al. (1997), who attributed such phenomena to $^{15}$NO$_2^-$ impurity in $^{15}$NO$_3^-$ tracer, and $^{15}$NO$_2^-$ rather than $^{15}$NO$_3^-$ underwent rapid chemical reduction to N$_2$O. However, the amount of $^{15}$NO$_2^-$ impurity in the $^{15}$NO$_3^-$ tracer used in this study was 80 ppm, which was not sufficient to enrich the initial $^{15}$N-N$_2$O to the observed level. The reason for this initial enrichment was not fully understood and requires further investigation.

Potential rates of N$_2$O production from NH$_4^+$ and NO$_3^-$ under controlled environmental conditions were shown in this study. To further investigate in situ N$_2$O production in natural salt marshes, nonintrusive methods such as in situ incubations coupled with flux chambers (Moseman-Valtierra et al. 2011), or evaluation of N$_2$O isotopic signatures from different production pathways, would be necessary.

5. Conclusion

Coastal salt marshes play an important role in the removal of land-derived excess nitrogen via several microbial processes that produce N$_2$O. Using $^{15}$N tracer incubations and numerical modeling, the availabilities of inorganic nitrogen and oxygen and nitrogen loading were the controlling factors of N$_2$O production and consumption. NO$_3^-$ was the major nitrogen substrate for N$_2$O production under high NO$_3^-$ concentrations, and NH$_4^+$ was the dominant nitrogen source under low NO$_3^-$ concentrations. Oxygen was critical in regulating N$_2$O consumption, which was enhanced in incubations under anoxic headspace. Decadal-scale fertilization increased sediment NH$_4^+$ and NO$_3^-$ concentrations; plots with the highest fertilization had significantly higher rates of N$_2$O production and consumption than control. Therefore, increasing anthropogenic nitrogen loading will increase nitrogen substrate availabilities and nitrogen transformation rates, and thus, N$_2$O flux from salt marshes is likely to increase. Short-term, high fluxes of N$_2$O are possible if N$_2$O production and consumption decouple as a consequence of coastal eutrophication.

Acknowledgments. The authors are indebted to the owners of salt marsh parcels, Salt Pond Sanctuaries and Dr. E. F. X. Hughes, for allowing us to have access to the experimental plots within their properties. We gratefully acknowledge those responsible for the establishment of the long-term experimental plots, which made these experiments possible. The fertilization experiment at the Great Sippewissett Marsh started with a collaboration between Ivan Valiela and John Teal. In recent years, Brian Howes and Dale Goehringer have maintained the fertilization plots, with funding support from the National Science Foundation (NSF; OCE-0453292, DEB-0516430 to I. Valiela). The authors thank John Angell and Patrick Kearns for their support during sample collection.

In the preparation of this manuscript, the authors acknowledge Frederik Simons for significant advice on numerical modeling and optimization, and François Morel and Daniel Sigman for their
helpful advice and discussions on this manuscript. The manuscript was greatly improved by suggestions from two anonymous reviewers. This work was funded by the NSF (DEB-1019624 to BBW and JLB).

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Received: 19 August 2014; revised: 7 April 2015.